



# APOE Genotypes Associate With Cognitive Performance but Not Cerebral Structure: Diabetes Heart Study MIND

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## OBJECTIVE

Dementia is a debilitating illness with a disproportionate burden in patients with type 2 diabetes (T2D). Among the contributors, genetic variation at the apolipoprotein E locus (*APOE*) is posited to convey a strong effect. This study compared and contrasted the association of *APOE* with cognitive performance and cerebral structure in the setting of T2D.

## RESEARCH DESIGN AND METHODS

European Americans from the Diabetes Heart Study (DHS) MIND ( $n = 754$ ) and African Americans from the African American (AA)-DHS MIND ( $n = 517$ ) were examined. The cognitive battery assessed executive function, memory, and global cognition, and brain MRI was performed.

## RESULTS

In European Americans and African Americans, the *APOE* E4 risk haplotype group was associated with poorer performance on the modified Mini-Mental Status Examination ( $P < 0.017$ ), a measure of global cognition. In contrast to the literature, the *APOE* E2 haplotype group, which was overrepresented in these participants with T2D, was associated with poorer Rey Auditory Verbal Learning Test performance ( $P < 0.032$ ). Nominal associations between *APOE* haplotype groups and MRI-determined cerebral structure were observed.

## CONCLUSIONS

Compared with *APOE* E3 carriers, E2 and E4 carriers performed worse in the cognitive domains of memory and global cognition. Identification of genetic contributors remains critical to understanding new pathways to prevent and treat dementia in the setting of T2D.

Type 2 diabetes (T2D) increases the overall risk of dementia by 50–60%, including the most commonly diagnosed forms of dementia: Alzheimer disease and vascular dementia (1–3). T2D is also associated with subclinical changes in cognition and brain structure and function that may increase the risk for dementia, including subclinical cognitive decline, lower regional brain volumes, increased burden of white matter lesions (WMLs), and altered cerebral metabolism and neural activity. Although the overall incident rates of dementia are reportedly in decline (4), the incidence of dementia in patients with T2D continues to increase. The precise mechanisms by which T2D increases the risk for dementia are not known, but

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substantial evidence links dementia risk to vascular disease and dysregulation of glucose and insulin, all of which are core features of T2D (5).

In addition, prevalence of dementia varies by ethnicity, with higher risk reported in non-Europeans (6), populations with disproportionate burdens of T2D (7). Recently, the Alzheimer's Association highlighted the elevated risk of dementia for African Americans in the U.S. Cultural factors may contribute to increased risk of both dementia and T2D in African Americans, including socioeconomic status and historical disparities in educational access. In this study, we investigate another important factor: genetic ancestry.

The most consistently observed genetic contributor to late-onset dementia is the apolipoprotein E (apoE) gene (*APOE*). The apoE protein exists in three common isoforms, apoE2, apoE3, and apoE4, encoded by three alleles,  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4, respectively. The *APOE*  $\epsilon$ 4 allele has been linked to an increased risk of Alzheimer disease and vascular dementia, and evidence is converging that this is true in different ethnicities, including African Americans, although allele frequencies may vary in different ethnic populations. The *APOE*  $\epsilon$ 3 allele is the most common allele in most ethnic groups, including Americans of European and African ancestry, and the  $\epsilon$ 2 allele is thought to confer some protective effects from Alzheimer disease (8). In addition to increasing the risk for dementia, evidence is emerging that T2D may interact with *APOE*  $\epsilon$ 4 allele status to further increase dementia risk, although whether that is true in African American populations is not known.

The objective of this study was to evaluate the association of *APOE* with cognitive performance and cerebral structure in European Americans and African Americans with T2D from the Diabetes Heart Study (DHS) MIND and African American (AA)-DHS MIND, respectively. These cohorts underwent cognitive testing and cerebral imaging. The results have the potential to identify ethnic-specific differences in *APOE* associations with cognitive decline and cerebral structure.

## RESEARCH DESIGN AND METHODS

### Subjects

Self-described European Americans and African Americans with T2D were

recruited at Wake Forest School of Medicine (WFSM) to the DHS MIND (9) and the AA-DHS MIND (10), respectively. The DHS is a cross-sectional study of European American and African American families with siblings concordant for T2D. AA-DHS was initiated after DHS and enrolled unrelated African Americans with T2D. MIND study objectives were to improve understanding of risk factors for cognitive impairment in T2D and assess cerebral architecture using MRI, contrasting results in European Americans and African Americans. This analysis included 754 European Americans and 517 African Americans with measures of cognitive function and brain MRI.

Study participants had a diagnosis of T2D with disease onset after the age of 30 years (and absence of diabetic ketoacidosis) in the setting of 1) active medical treatment for diabetes (insulin and/or oral hypoglycemic agents), 2) fasting blood glucose  $\geq 126$  mg/dL or nonfasting blood glucose  $\geq 200$  mg/dL, or 3) hemoglobin (Hb) A<sub>1c</sub>  $\geq 6.5\%$ . Examinations were performed in the WFSM Clinical Research Unit. In addition to recording medical and education histories, vital signs, and current medications, subjects underwent fasting measurements of serum creatinine, blood urea nitrogen, thyroid-stimulating hormone, vitamin B<sub>12</sub> levels, and urine albumin-to-creatinine ratio (LabCorp, Burlington, NC). After a midmorning snack, cognitive testing and cerebral MRI were performed. This study was approved by the WFSM Institutional Review Board and adhered to the Declaration of Helsinki. Written informed consent was obtained from all study participants.

### Cognitive Testing

Participants completed an ~45-min cognitive battery that tested global cognition, memory, processing speed, and executive function (11). Interviewers were trained, certified, and subsequently assessed for quality control in all cognitive tests by a study investigator (K.M.S.). Global cognition was assessed on a 100-point scale with the modified Mini-Mental State Examination (3MSE) (12). Learning and memory were assessed with the Rey Auditory Verbal Learning Test (RAVLT), a word-list recall task reported here as the total number of words recalled across the first five tests (13). Executive function was assessed with the

total number correct on Digit Symbol Coding Task (a measure of processing speed and to a lesser extent, working memory) (14), the Stroop Test (15) (measuring response inhibition, specifically reported here as interference), and phonemic fluency (reported here as the number of words generated for the letter F). Testing was performed in a quiet room after the midmorning snack.

### Cerebral MRI

#### MRI Acquisition

For DHS MIND and AA-DHS MIND the initial 643 and 61 scans, respectively, were performed on a 1.5-Tesla (T) EXCITE HD scanner with twin-speed gradients using a neurovascular head coil (GE Healthcare, Milwaukee, WI). High-resolution T1 anatomic images were obtained using a three-dimensional (3D) volumetric inversion recovery spoiled gradient recalled acquisition in steady state recalled sequence (repetition time [TR], 7.36 ms; echo time [TE], 2.02 ms; inversion time [TI], 600 ms; flip angle [FA], 20°; 124 slices at field of view, 24 cm; matrix size, 256 × 256; 1.5-mm slice thickness). Fluid-attenuated inversion recovery images were acquired in the axial plane (TR, 8,002 ms; TE, 101.29 ms; TI, 2,000 ms; FA, 90°; field of view, 24 cm; matrix size, 256 × 256; 3-mm slice thickness). Because of a change in scanners at the WFSM Center for Biomolecular Imaging, the subsequent 12 DHS MIND and 418 AA-DHS MIND scans were performed on 3.0-T scanners (either a Signa EXCITE scanner [GE Healthcare] or a Skyra MRI scanner [Siemens Healthcare, Erlangen, Germany]) using a high-resolution 20-channel head/neck coil. T1-weighted anatomic images were obtained using a 3D volumetric magnetization-prepared rapid acquisition gradient echo sequence (TR, 2,300 ms; TE, 2.99 ms; TI, 900 ms; FA, 9°; 192 slices; voxel dimension, 0.97 × 0.97 × 1 mm). Fluid-attenuated inversion recovery images were acquired using a 3D SPACE inversion recovery sequence (TR, 6,000 ms; TE, 283 ms; TI, 2,200 ms; FA, 120°; 160 slices; voxel dimensions, 1.1 × 1.1 × 1 mm).

#### Image Segmentation

Structural T1 images were segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). Images were then normalized to the Montreal Neurological Institute imaging space and modulated with the Jacobian

determinants (nonlinear components only) of the warping procedure to generate volumetric tissue maps using the Dartel high-dimensional warping and the SPM8 (16) new segment procedure, as implemented in the VBM8 toolbox (<http://dbm.neuro.uni-jena.de/vbm.html>). The modulation step did not include the affine component of the normalization parameters, thereby correcting the volumetric tissue maps for total intracranial volume (ICV). Total GM volume (GMV), WM volume (WMV), CSF volume (CSFV), and ICV (GMV + WMV + CSFV) were determined from the VBM8 automated segmentation procedure, which outputs a text file with values for native space total GMV, WMV, and CSFV. Additional measures based on the region of interest (ROI) were generated for the right and left hippocampus using the automated anatomical labeling atlas (17), as implemented in the WFU\_Pickatlas (18). The automated anatomical labeling atlas hippocampal ROI is not specific to the GM; it encompasses GM, WM, and CSF tissue types. The hippocampal ROIs (right and left) were applied to the modulated GM and WM volumetric tissue maps to generate hippocampal GMV and hippocampal WMV. All volumes were reported in cubic centimeters by summing the voxel values, multiplying by the voxel volume (in cubic millimeters), and dividing by 1,000 adjusted for ICV. Results were similar between the left and right hippocampus; therefore, we present only the total hippocampal volumes.

#### WML Segmentation

WML segmentation was performed using the lesion segmentation toolbox (19) for SPM8 at a threshold ( $k$ ) of 0.25. The lesion segmentation toolbox has been validated against expert manual segmentation as well as identifying the optimum thresholds (20). Normalization to Montreal Neurological Institute space was accomplished by coregistration with the structural T1 and applying the normalization parameters computed in the VBM8 segmentation procedure. WML volume (WMLV) was determined by summing the binary lesion maps and multiplying by the voxel volume, and values are reported in cubic centimeters.

#### Genotyping

Total genomic DNA was purified from whole-blood samples obtained from MIND participants using the PUREGENE

DNA isolation kit (Gentra, Inc., Minneapolis, MN). DNA concentration was quantified using standardized fluorometric readings on a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech Inc., San Francisco, CA). *APOE* haplotype status was determined from direct genotyping of two single nucleotide polymorphisms (SNPs): rs429358 (hg19, chr19:45411941, T>C) and rs7412 (hg19, chr19:45412079, C>T).

In the DHS MIND, genotypes were determined using custom assays on a MassARRAY SNP Genotyping System (Sequenom Inc., San Diego, CA) following standard protocols, as previously reported (21). Genotype calls were manually reviewed using MassArray Typer v3.4 software (Sequenom Inc.). In the AA-DHS MIND, genotypes were determined using TaqMan technology (Life Technologies Corp., Grand Island, NY), following the manufacturer's protocol. Predesigned assays were ordered for genotyping on an Applied Biosystems ViiA7 instrument. Included in the genotyping were 47 quality control samples that served as blind duplicates and allowed for evaluation of genotyping accuracy. The concordance rate for these blind duplicates was 100%. To ensure comparability across platforms, a subset of AA-DHS MIND samples (18 for rs7412 and 17 for rs429358) were genotyped on Sequenom and with TaqMan technology and resulted in 100% concordance.

#### Statistical Analysis

Two coding variants in the human *APOE* gene, rs429358 (Cys130Arg) and rs7412 (Arg176Cys), define the apoE protein as three isoforms (E2, E3, and E4). Haplotypes were used to define protein isoforms: apoE2 ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ ), apoE3 ( $\epsilon 3/\epsilon 3$ ; reference), and apoE4 ( $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 4$ ,  $\epsilon 4/\epsilon 4$ ) by using a codominant Mendelian inheritance model. Outcome variables included serum lipid concentrations, cognitive testing, and MRI measures. Sample means and SDs were computed for continuous variables, and proportions were calculated for categorical characteristics. Statistical significance was defined as a  $P$  value  $<0.05$  given the a priori evidence of association for this locus with dementia.

As stated, DHS MIND enrolled sibling pairs and AA-DHS MIND enrolled unrelated individuals. The statistical analysis varied by study to account for differences in the

study design. In DHS MIND, the unadjusted comparisons across *APOE* haplotypes were performed using the marginal model incorporating generalized estimating equations. This model accounted for familial correlation using a sandwich estimator of the variance assuming exchangeable correlation. Box-Cox transformations were applied to lipid and MRI outcomes as needed. Marginal models with identity link and normal distribution were fitted across *APOE* haplotype groups for lipid and MRI measures. For lipids measures, the model was adjusted for age, sex, BMI, HbA<sub>1c</sub>, statins, cardiovascular disease (CVD; defined as prior stroke, transient ischemic attack, myocardial infarction, and coronary artery bypass grafting, angioplasty, or stenting), smoking, and hypertension. For MRI measures, the model was adjusted for age, sex, BMI, HbA<sub>1c</sub>, statins, CVD, smoking, hypertension, scanner (1.5-T or 3.0-T), and total ICV for WMV, WMLV, GMV, and CSFV. The same model was used for hippocampal WMV and hippocampal GMV without adjustment for ICV. For cognitive testing measures, marginal models with log-link and Poisson distribution were fitted and compared across *APOE* haplotypes adjusting for age, sex, BMI, HbA<sub>1c</sub>, statins, CVD, smoking, hypertension, and educational attainment. The regression coefficient estimates and their associated SEs are reported.

In AA-DHS MIND, unadjusted comparisons across *APOE* haplotypes were based on the Kruskal-Wallis test. Association between the *APOE* haplotypes and lipid and MRI outcomes were tested using the linear model framework where the dependent variable was transformed as needed. For lipid outcomes, models were adjusted for age, sex, BMI, statins, HbA<sub>1c</sub>, CVD, smoking, hypertension, and African ancestry proportion (determined in a genome-wide association study). Models for MRI variables (WMV, WMLV, GMV, and CSFV) were adjusted for age, sex, BMI, statins, HbA<sub>1c</sub>, CVD, smoking, hypertension, African ancestry proportion, scanner (1.5-T or 3.0-T), and total ICV.

For hippocampal WMV and GMV, the adjustment was the same, except that total ICV was not adjusted. Generalized linear models were fitted for the cognitive function variables using log-link and a negative binomial distribution to account for observed overdispersion. Models were adjusted for age, sex, BMI,

lipid-lowering medication, HbA<sub>1c</sub>, CVD, smoking, hypertension, educational attainment, and African ancestry proportion. The regression coefficient estimates and their associated SEs were reported.

## RESULTS

Demographic and clinical characteristics of the DHS MIND and AA-DHS MIND cohorts are summarized in Table 1 collapsed by haplotype carrier status and in Supplementary Table 1 by haplogenotype status. DHS MIND consisted of 754 European Americans with T2D from 539 families (368 male, 386 female) with a mean (SD) age of 65.9 (9.8) years, BMI 33.0 (6.8) kg/m<sup>2</sup>, and HbA<sub>1c</sub> 7.5% (1.4); 89% had hypertension, and 49.8%

were receiving lipid-lowering medications. No differences were observed in age, sex, HbA<sub>1c</sub>, smoking, hypertension, statins, or prior CVD based on APOE haplotype grouping. BMI was modestly higher among the APOE E2 group ( $P = 0.043$ ). AA-DHS MIND consisted of 516 African Americans with T2D (202 male, 314 female) with a mean (SD) age of 58.6 (9.6) years, BMI 35.3 (8.5) kg/m<sup>2</sup>, and HbA<sub>1c</sub> of 8.1% (2.0); 86% had hypertension, and 51.5% were receiving statins. No differences were observed in age, sex, BMI, HbA<sub>1c</sub>, smoking, hypertension, statins, or CVD among APOE haplotype groups. Comparatively, both populations had a similar diabetes duration (>10 years), relatively good

glycemic control (HbA<sub>1c</sub> <8.1%), lack of advanced kidney disease, frequent use of statins (>50%), and relatively controlled blood pressure.

Among European Americans, overall isoform frequencies were 0.13 for ε2, 0.76 for ε3, and 0.11 for ε4, and the overall haplotype frequencies were 0.019 for ε2/ε2, 0.186 for ε2/ε3, 0.031 for ε2/ε4, 0.572 for ε3/ε3, 0.186 for ε3/ε4, and 0.008 for ε4/ε4. Among African Americans, overall isoform frequencies were 0.17 for ε2, 0.45 for ε3, and 0.38 for ε4, and the overall haplotype frequencies were 0.013 for ε2/ε2, 0.157 for ε2/ε3, 0.048 for ε2/ε4, 0.453 for ε3/ε3, 0.277 for ε3/ε4, and 0.052 for ε4/ε4.

**Table 1—Demographic characteristics of study participants**

	APOE haplotype status				
	E2	E3	E4	Overall	P value
European Americans in DHS MIND					
Demographic variables					
N	154	431	169	754	
Age (years)	64.6 (10.5)	66.2 (9.5)	66.0 (9.8)	65.9 (9.8)	0.30
Sex (% female)	50.0	50.8	53.3	51.2	0.89
BMI (kg/m <sup>2</sup> )	34.2 (6.6)	32.7 (6.5)	32.7 (7.5)	33.0 (6.8)	0.043
Diabetes duration (years)	15.6 (8.3)	15.5 (7.3)	15.0 (8.3)	15.4 (7.7)	0.60
HbA <sub>1c</sub> , % (mmol/mol)	7.5 (58)	7.5 (58)	7.5 (58)	7.5 (58)	0.54
Smoking (%)					0.76
Never	42.8	44.6	49.4	45.3	
Current or former	57.2	55.4	50.6	54.8	
Hypertension (%)	86.4	90.7	87.0	89.0	0.21
Lipids medication (%)	46.1	49.3	54.4	49.8	0.30
Prior CVD (%)	30.6	40.5	39.6	38.3	0.079
Cognitive performance variables					
3MSE	91.1 (7.0)	91.0 (6.9)	90.1 (8.5)	90.8 (7.3)	0.24
Stroop Task	33.7 (14.8)	35.7 (22.0)	32.6 (18.6)	34.6 (20.0)	0.14
Digit Symbol Coding Task	50.5 (16.4)	49.5 (16.5)	49.5 (15.2)	49.7 (16.2)	0.59
RAVLT	40.3 (9.8)	40.9 (10.2)	39.9 (11.1)	40.6 (10.3)	0.17
Phonemic fluency	10.0 (4.6)	10.2 (4.2)	10.9 (4.1)	10.4 (4.3)	0.16
African Americans in AA-DHS MIND					
Demographic variables					
N	89	237	190	516	
Age (years)	57.3 (9.9)	59.1 (9.8)	58.6 (9.3)	58.6 (9.6)	0.56
Sex (% female)	59.6	57.0	66.5	60.9	0.13
BMI (kg/m <sup>2</sup> )	35.3 (8.3)	34.9 (8.7)	35.7 (8.4)	35.3 (8.5)	0.39
Diabetes duration (years)	12.2 (6.7)	13.5 (8.0)	13.0 (7.6)	13.1 (7.7)	0.49
HbA <sub>1c</sub> , % (mmol/mol)	8.4 (68)	8.0 (64)	8.0 (64)	8.1 (65)	0.33
Smoking (%)					0.82
Never	46.1	43.6	46.6	45.1	
Current or former	53.9	56.4	53.4	54.9	
Hypertension (%)	86.0	85.0	87.2	86.0	0.82
Lipids medication (%)	38.8	58.1	49.6	51.5	0.060
Prior CVD (%)	21.3	23.6	22.1	22.7	0.88
Cognitive performance variables					
3MSE	84.3 (8.8)	86.3 (9.1)	86.0 (7.9)	85.8 (8.6)	0.090
Stroop Task	38.74 (20.5)	42.2 (20.4)	40.6 (16.0)	41.0 (18.9)	0.59
Digit Symbol Coding Task	50.8 (17.3)	49.1 (16.4)	50.4 (16.0)	49.9 (16.4)	0.64
RAVLT	35.5 (9.3)	37.2 (8.5)	38.7 (8.7)	37.5 (8.8)	0.030
Phonemic fluency	11.0 (4.5)	10.7 (5.3)	10.9 (4.2)	10.9 (4.5)	0.72

Values are presented as mean (SD), unless otherwise noted.

Associations between *APOE* haplotype groups and lipid profile parameters included total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations overall by ethnicity and stratified by use of statins (Supplementary Table 2). In European Americans, the *APOE* E2 haplotype group was associated with lower LDL cholesterol concentrations ( $\beta = -0.092$ ,  $P = 0.020$ ), a result driven by participants not taking statins ( $\beta = -0.10$ ,  $P = 0.024$ ). The strongest association in participants taking a statin was with lower triglyceride concentrations in the *APOE* E2 haplotype group ( $\beta = -0.012$ ,  $P = 3.67 \times 10^{-6}$ ). Overall, the *APOE* E4 haplotype trended toward increased LDL cholesterol ( $\beta = 0.041$ ,  $P = 0.093$ ) and HDL cholesterol ( $\beta = 0.00032$ ,  $P = 0.067$ ). In African Americans, the *APOE* E2 haplotype group showed a trend toward an association with lower levels of LDL cholesterol ( $\beta = -0.098$ ,  $P = 0.092$ ), consistent with the findings in European Americans.

Results of the associations between *APOE* haplotypes and measures of cognitive performance are presented in Table 2. Among European Americans, the *APOE* E4 risk haplotype group was associated with poorer performance on the 3MSE ( $\beta = -0.16$ ,  $P = 0.017$ ) and RAVLT ( $\beta = -0.055$ ,  $P = 0.0089$ ) compared with the E3 haplotype group. The *APOE* E2 haplotype group, posited to convey protection from Alzheimer disease, was also associated with poorer RAVLT performance ( $\beta = -0.046$ ,  $P = 0.032$ ) and phonemic fluency ( $\beta = -0.082$ ,  $P = 0.047$ ) compared with the E3 haplotype group. Among African Americans, and consistent with findings in European Americans, the *APOE* E4 risk haplotype group was associated with poorer performance on the 3MSE ( $\beta = -0.14$ ,  $P = 0.014$ ), and the *APOE* E2 haplotype group was associated with poorer performance on the RAVLT ( $\beta = -0.059$ ,  $P = 0.027$ ) and 3MSE ( $\beta = -0.22$ ,  $P = 0.0012$ ) compared with the E3 haplotype group. Subgroup analysis revealed that these results were driven by the more common haplogenotypes (Supplementary Table 3). These associations persisted after accounting for the degree of physical activity undertaken by study participants (Supplementary Table 4) and use of waist circumference, rather than BMI, as a proxy of abdominal adiposity (Supplementary Table 5).

**Table 2—Association of *APOE* haplotypes with cognitive performance by ethnic group**

Trait	<i>APOE</i> haplotype	European Americans			African Americans		
		$\beta^1$	SE <sup>2</sup>	<i>P</i> value <sup>3</sup>	$\beta^1$	SE <sup>2</sup>	<i>P</i> value <sup>4</sup>
3MSE	E2	−0.11	0.061	0.065	−0.22	0.071	<b>0.0012</b>
	E4	−0.16	0.069	<b>0.017</b>	−0.14	0.055	<b>0.014</b>
Stroop Task	E2	−0.0022	0.043	0.96	−0.034	0.050	0.47
	E4	−0.074	0.052	0.16	−0.012	0.038	0.76
Digit Symbol Coding Task	E2	−0.038	0.025	0.13	−0.0038	0.036	0.92
	E4	−0.035	0.023	0.13	−0.017	0.028	0.54
RAVLT	E2	−0.046	0.021	<b>0.032</b>	−0.059	0.026	<b>0.027</b>
	E4	−0.055	0.021	<b>0.0089</b>	−0.00030	0.020	0.99
Phonemic fluency	E2	−0.082	0.041	<b>0.047</b>	−0.057	0.053	0.28
	E4	0.045	0.036	0.21	−0.046	0.040	0.25

<sup>1</sup>The  $\beta$ -estimate for analysis using the *APOE* E3 haplotype as reference. <sup>2</sup>SE for the parameter estimate for analysis using the *APOE* E3 haplotype as reference. <sup>3</sup>Adjusted for age, sex, BMI, HbA<sub>1c</sub>, statin medication, CVD status, smoking status, hypertension status, and educational attainment. Values of  $P < 0.05$  are in bold. <sup>4</sup>Adjusted for age, sex, BMI, lipid medication, HbA<sub>1c</sub>, CVD status, smoking status, hypertension status, educational attainment, and African ancestry proportion. Values of  $P < 0.05$  are in bold.

Associations between *APOE* haplotype groups and MRI-determined cerebral structure are presented in Table 3. Among the brain regions examined, compared with the *APOE* E3 haplotype group, the *APOE* E4 haplotype group was associated with an increase in WMLV in European Americans ( $\beta = 2.11$ ,  $P = 0.045$ ). No associations were observed in the African American cohort.

## CONCLUSIONS

T2D and non-European ancestry both elevate risk for dementia and cognitive

decline. This analysis examined the effects of *APOE* on cognition, a common risk allele for late-onset Alzheimer disease, in people with T2D of European and recent African ancestry. Results show that the *APOE* E4 haplotype was associated with lower cognitive scores in a cohort with T2D. The association between cognitive performance and *APOE* in people with T2D was similar in magnitude and direction for participants with both European and African ancestry; for both groups, the *APOE* E4 haplotype was associated with lower

**Table 3—Association of *APOE* haplotypes with MRI-based cerebral structure by ethnic group**

Trait	<i>APOE</i> haplotype	European Americans			African Americans		
		$\beta^1$	SE <sup>2</sup>	<i>P</i> value <sup>3</sup>	$\beta^1$	SE <sup>2</sup>	<i>P</i> value <sup>4</sup>
WMV	E2	0.74	3.01	0.81	7.47	8.48	0.38
	E4	−3.83	2.69	0.15	6.75	6.51	0.30
Hippocampal WMV	E2	0.0019	0.032	0.95	0.018	0.016	0.24
	E4	−0.050	0.031	0.11	0.015	0.012	0.20
WMLV	E2	0.37	0.75	0.62	−0.13	0.20	0.51
	E4	2.11	1.05	<b>0.045</b>	−0.22	0.15	0.16
GMV	E2	−3.98	2.88	0.17	−1.7E−03	3.7E−03	0.65
	E4	1.24	2.56	0.63	−2.3E−04	2.8E−03	0.94
Hippocampal GMV	E2	0.094	0.11	0.39	−0.27	0.33	0.42
	E4	0.013	0.11	0.90	0.095	0.26	0.71
CSFV	E2	3.14	2.50	0.21	−20539.52	19977.13	0.30
	E4	2.41	2.49	0.33	−20686.23	15328.92	0.18

<sup>1</sup>The  $\beta$ -estimate for analysis using the *APOE* E3 haplotype as reference. <sup>2</sup>SE for the parameter estimate for analysis using the *APOE* E3 haplotype as reference. <sup>3</sup>WMV, WMLV, GMV, and CSFV were adjusted for age, sex, BMI, HbA<sub>1c</sub>, statin medication, CVD, smoking, hypertension, scanner, and total ICV; hippocampal WMV and GMV were adjusted for age, sex, BMI, HbA<sub>1c</sub>, statin medication, CVD, smoking, hypertension, and scanner. Values of  $P < 0.05$  are in bold. <sup>4</sup>Adjusted for age, sex, BMI, HbA<sub>1c</sub>, statin medication, CVD, smoking, hypertension, and African ancestry proportion.



scores on the 3MSE, a test of global cognition, and the RAVLT, a test of memory that is an important predictor in the transition to Alzheimer disease (22). Unexpectedly, the *APOE* E2 haplotype was associated with poorer cognitive performance, rather than a protective factor, for both European American and African American participants with T2D.

In contrast to associations with cognitive performance, associations between brain MRI measures (GMV, WMV, CSF, WMLs, and hippocampal volume) were nominal or absent. The only association observed was between *APOE* E4 haplotype and WMLV in European Americans. Similar results were observed in large meta-analysis efforts focused primarily in the European-derived population with the additional finding that *APOE* E2 was associated with increasing white matter hyperintensity load (23). Analyses in other ethnic groups have been limited, affecting the generalizability of these findings.

Consistent with the literature (24), lower LDL cholesterol concentration was present in European Americans with the *APOE* E2 haplotype ( $\beta = -0.092$ ,  $P = 0.020$ ), with trends of a similar magnitude and in the same direction for African Americans ( $\beta = -0.098$ ,  $P = 0.092$ ). Quantitatively, this translates to an average 2.7 mg/dL decrease among the *APOE* E2 haplotype group compared with E3 ( $99.6 \pm 38.3$  mg/dL and  $102.3 \pm 31.4$  mg/dL, respectively) among European Americans.

Although most of the published literature on dementia focuses on risk attributed to the *APOE* E4 haplotype, associations with poorer cognitive performance were also observed for *APOE* E2 in this sample affected by T2D. The E2 haplotype group was also more common in these cohorts with diabetes compared with other multiethnic large-scale meta-analyses (25). Among European Americans with T2D, *APOE* E2 was associated with poorer memory based on the RAVLT and with reduced executive function based on tests of phonemic fluency. Notably, these associations were not observed in the African American population, despite similar sample sizes for E4 carriers (Table 1). The associations with RAVLT were the most consistent across the ethnic groups. Evidence is limited, but these findings support previous studies that have noted an increased

percentage of *APOE* E2 allele carriers in people with diabetes and in African Americans (26,27). Given the increased frequency of the E2 haplotype in the population with diabetes, further efforts are needed to characterize the effects of the component haplogentotypes, E2E2 and E2E3, especially E2E2, which is relatively infrequent in the population (Supplementary Table 1). In addition, this subgrouping could help further characterize potential ethnic-specific effects of *APOE* variation on cognitive outcomes.

Strengths of the DHS MIND and AA-DHS MIND studies are the recruitment of a well-characterized biethnic cohort affected by T2D, including a diverse collection of sophisticated CVD phenotypes, a range of cognitive domains, and MRI brain imaging. However, this study is not without limitations. Evaluation of European Americans and African Americans allowed for a contrast of findings; however, African Americans are known to perform worse on a variety of cognitive tests compared with European Americans, potentially because of differences in education (28,29), socioeconomic factors, and cognitive risk factors (North Carolina is in the stroke belt) (30). Moreover, health conditions (e.g., sleep-disordered breathing) (31) not assessed in this study and medication usage (which varies over time) may alter the outcome of cognitive testing. In addition, these participants volunteered to be in a research study, and study volunteers are often healthier than the general population. Finally, these were cross-sectional studies of late middle-aged cohorts and are therefore unable to determine definitively whether the *APOE* alleles are associated with within-person cognitive changes that manifest their presence more clearly in older age. Although African Americans were younger than European Americans, durations of T2D were similar between groups.

In conclusion, these results demonstrate that variation in several cognitive domains were associated with the *APOE* locus, findings independent from brain changes based on MRI. Compared with noncarriers, *APOE* E2 and E4 carriers consistently performed worse in the cognitive domains of memory and global cognition. Identification of genetic contributors remains critical to understanding

new pathways to prevent and treat dementia.

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